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Endocannabinoids Inhibit the Growth of Free-Living Amoebae[▽]

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The cannabinoid Δ^9-tetrahydrocannabinol inhibits the growth of some pathogenic amoebae *in vitro* and exacerbates amoebic encephalitis in animal models. However, the effects of endogenous cannabinoids on amoebae remain unknown. Therefore, we tested several endocannabinoids (*N*-acyl ethanolamines and 2-*O*-acyl glycerol) on different genera of amoebae. The results showed that all of the endocannabinoids tested inhibit amoebic growth at subpharmacological doses, with 50% inhibitory concentrations ranging from 15 to 20 μM. A nonhydrolyzable endocannabinoid had similar effects, showing that the inhibition seen results from endocannabinoids *per se* rather than from a catabolic product.

Some free-living amoebae, such as members of the genera *Acanthamoeba* and *Naegleria*, are responsible for severe encephalitis and dermatitis with increasing prevalence, especially in immunocompromised patients (3, 11, 12). In addition to numerous side effects, such as seizures, nausea, and vomiting, granulomatous amebic encephalitis (GAE) is usually fatal to patients who do not have access to efficient therapeutic approaches (11). The plant-derived psychoactive cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) was shown to inhibit the growth of the pathogen *Naegleria fowleri in vitro* (14). However, this cannabinoid also shows immunosuppressive activity and exacerbates encephalitis due to opportunistic amoebae in a mouse model of GAE by inhibiting macrophage-like cell activity (2, 3). Unlike Δ^9-THC, the endogenous cannabinoid 2-*O*-arachidonoyl glycerol (2-*O*-AG; one of the two prototypic endocannabinoids, along with *N*-arachidonoyl ethanolamine) activates immune responses, including chemotaxis of macrophages and microglial cells (3, 17), but its effects on free-living amoebae remain unknown. Some observations showed that amoebae display an active endocannabinoid system inasmuch as *N*-acyl phosphatidylethanolamine (the precursor of the *N*-acyl ethanolamine endocannabinoids) is expressed in a regulated fashion during the development of *Dictyostelium discoideum* amoeba (8). Moreover, arachidonoyl ethanolamine is metabolized in *Tetrahymena pyriformis* through fatty acid amide hydrolase activity (7, 9). Endocannabinoids may therefore impact amoebic cell fate. Therefore, we tested the effects of several endocannabinoids (*N*-arachidonoyl ethanolamine, 2-*O*-AG, and 2-*O*-AG ether from Cayman Chemical, Ann Arbor, MI) on different genera of amoebae.

The amoebae used in this study were *Acanthamoeba castellanii* (By 02.2.4), *Hartmannella vermiformis* (Ax.5.2e4b), and *Willaertia magna* c2c Maky (ATCC PTA-7824). Cells were grown at 30°C on a lawn of *Escherichia coli* on nonnutrient agar and were established in axenic culture at 37°C in serum casein glucose yeast extract medium (SCGYEM) (4).

On day 0 of experiments, amoebae were seeded in culture tubes at a concentration of 2×10^5 cells/ml of SCGYEM which either contained various concentrations of endocannabinoids (see figure legend for details) or did not (controls). Endocannabinoids were added to the medium in an ethanol solution (ethanol at a final concentration of 0.05%). Control tubes also contained ethanol (vehicle). The tubes were placed at 37°C in a slanting position, and the cell concentrations were determined on a daily basis up to day 3 using a hemocytometer. Exposure of different free-living amoebae to *N*-arachidonoyl ethanolamine at a concentration of 10 μg/ml inhibited the growth of all of the amoebic species tested (Fig. 1A). After 3 days, the growth of *A. castellanii*, *W. magna*, and *H. vermiformis* was reduced by 68%, 58%, and 96%, respectively, compared to that of controls (the respective 50% inhibitory concentrations [IC_{50}s] of anandamide were ~17, 20, and 14 μM). Inhibition of amoebic growth was also observed with lower doses (2 and 5 μg/ml) of *N*-arachidonoyl ethanolamine (data not shown). This growth inhibition is further illustrated in Fig. 1B, showing that anandamide prevented the formation of amoebic cell monolayers. This effect was also obtained using endocannabinoids of the 2-*O*-acyl glycerol class (Fig. 1B). The IC_{50}s were in the same range as those of *N*-arachidonoyl ethanolamine. A toxic effect was observed at a concentration of 20 μg/ml, as shown by the decrease in viable cell concentrations compared to the number of cells seeded at day 0 (Fig. 1A). These effects may result from a direct action of endocannabinoids on amoebae or from the action of one of their catabolic products. In order to test that possibility, we compared the inhibitory effect of 2-*O*-AG with that of a nonhydrolyzable analogue (10), i.e., 2-*O*-AG ether (Table 1). The two molecules had similar effects on the various amoebae, although *H. vermiformis* proved to be more sensitive than the other two species at the concentration used (Table 1 compares the percentages of inhibition versus those obtained with the control). This observation strongly suggests that the inhibition of cell growth results from endocannabinoids *per se* rather than from a catabolic product.

These results showed that endocannabinoids displayed similar effects to the one reported for Δ^9-tetrahydrocannabinol on amoebic growth (14). Moreover, the inhibitory effects of endocannabinoids were observed at doses lower than or similar

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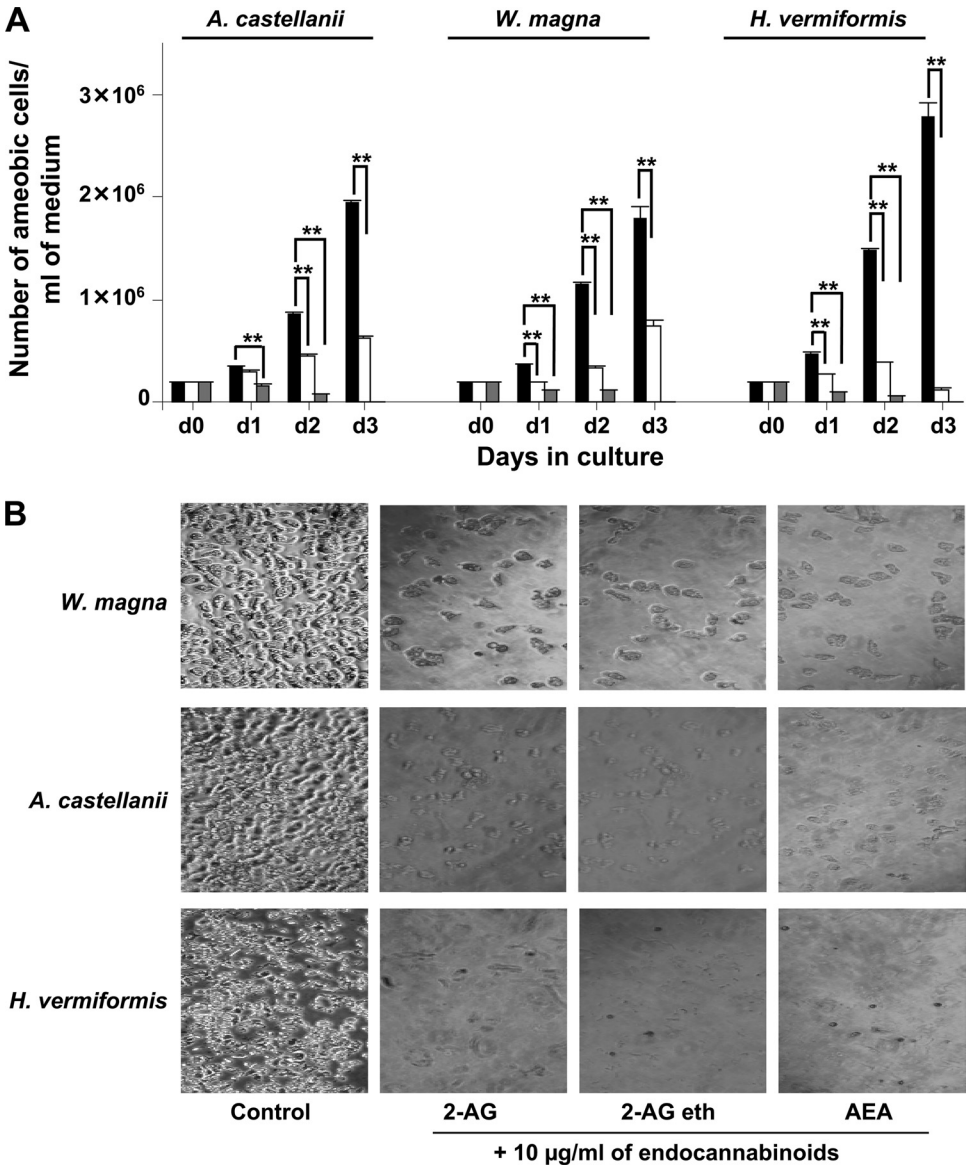

FIG. 1. Effects of endocannabinoids on amoebic growth. (A) Effect of *N*-arachidonoyl ethanolamine on amoebic growth. Cells were cultured for up to 3 days (d) either with (white bars, 10 μg/ml; gray bars, 20 μg/ml) or without (black bars) endocannabinoids. Cell concentrations were determined daily. The data are the average ± the standard error of the mean of four independent experiments performed in triplicate. Statistically significant differences (Student's *t* test) in the growth of amoebae cultured either with or without *N*-arachidonoyl ethanolamine are indicated (**, $P < 0.001$). (B) Representative phase-contrast images of the different amoebae cultured in 24-well plates either with or without different endocannabinoids taken after 3 days in culture. eth, ether, AEA, *N*-arachidonoyl ethanolamine.

TABLE 1. Effect of a nonhydrolyzable endocannabinoid on amoebic growth[a]

Treatment	No. of cells/ml (% decrease vs control)		
	A. castellanii	*W. magna*	*H. vermiformis*
Control (vehicle)	1,940 ± 25	1,790 ± 116	2,790 ± 130
2-*O*-AG	482 ± 14 (75.2)[b]	775 ± 42 (56.6)[b]	124 ± 15 (95.6)[b]
2-*O*-AG ether	477 ± 14 (75.4)[b]	783 ± 42 (56.2)[b]	340 ± 128 (87.8)[b]

[a] Cells were cultured for up to 3 days either with or without endocannabinoids (10 μg/ml). Cell concentrations were determined after 3 days in culture. The data are the average ± the standard error of the mean of cell concentrations obtained in four independent experiments performed in triplicate.

[b] $P < 0.001$ versus control (Student's *t* test).

to the pharmacological concentrations used in animal studies (16). These observations are particularly interesting since, unlike Δ^9-tetrahydrocannabinol, some endocannabinoids, such as 2-*O*-AG, are known to stimulate the immune response, including macrophage activity (3) (17). This property, taken together with our observations that endocannabinoids strongly inhibit the growth of free-living amoebae, suggests that modulation of the endocannabinoid system may be used in designing therapeutic approaches for pathogenic amoeba infections. Cannabinoids have already been used in the treatment of human glioma tumors with promising results (15). Endocannabinoids administrated *in vivo* would probably impact signaling through CB1 and CB2 receptors that are mainly expressed in the cen-

tral nervous system and immune cells, respectively, and neurobehavioral effects (spontaneous activity, hypothermia, antinociception, and catalepsy) would be expected, as shown in animals (6). However, unlike Δ^9-tetrahydrocannabinol, these pharmacological effects of endocannabinoids are completely dissipated by 30 min because of their different metabolism and half-life (1, 18). We do not know what mechanisms underlie the effect of endocannabinoids on the growth of free-living amoeba. Phylogenetic studies suggest that protozoans do not express CB1 and CB2 cannabinoid receptors (13). If this is true, it would imply that endocannabinoids act on free-living amoebas through different mechanisms. Indeed, endocannabinoids can modulate intracellular targets (reviewed in reference 17) and they can be metabolized to prostamides upon cyclooxygenase activity (19). A cyclooxygenase-like enzyme has recently been characterized in protozoa (5).

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